DISTRIBUTION AND CONCENTRATION OF POTENTIALLY TOXIC ELEMENTS IN *Casuarina* spp. ROOT TISSUES BY SCANNING ELECTRON MICROSCOPY AND X RAYS MICROANALYSIS.

[1]Sánchez-Viveros G., [2*]González-Chávez M.C., [3]Zavaleta-Mancera H.A. and [4*]Arenas-Alatorre J. [1,2,3]Colegio de Postgraduados, Montecillo Edo. de México. [4]Universidad Nacional Autónoma de México. *corresponding author

This research was partially supported by the Mexican grant SEMARNAT-CONACYT-CO-01-2002-739.

In a polluted soil with potentially toxic elements (PTEs), plants and associated microorganisms play a relevant role on direct and indirect contaminant remotion. The mechanisms involved may be by absortion, transformation or accumulation into tissues. Arbuscular mycorrhizal fungi (AMF), which associate to plant roots, are widely distributed (1). Extraradical hyphae may accumulate and control translocation of PTEs (2). Hence, AMF decrease plant toxicity. It is then interesting to analyze PTE-distribution and concentration in roots of plants that grow in polluted soils, in order to explain the interaction of AMF with plants and their strategies. Chemical analyses to determine PTEs content in biological samples involve samples destruction by digestion with concentrated acids; however PTEs localization *is situ* is not possible (3). In contrast, Scanning Electron Microscopy (SEM) with a X ray detector attached may provide information about localization and chemical composition of biological samples. Low vacuum SEM allows observation of samples in native state (dry or wet) with magnifications up to 500 000x with a minimum sample preparation, good field depth observations inside of the sample (~3μm), and possibility to combine with X ray microanalysis (for example EDS) getting digital images. Although, EDS has low detection limit (below 100 ppm), its use is frequent to establish EPTs distribution maps because of its facility to perform punctual chemical analysis.

The objective of this research was to study the distribution and relative concentration of EPTs in roots of *Cassuarina* spp. growing in polluted soils with heavy metals using Low vacuum SEM with an EDS attached. Transversal segments of secondary and tertiary AMF-colonized roots were fixed in 0.01 M phosphate buffer pH 7, containing 2.5% glutaraldehyde, 2% paraformaldehyde, and 1% sodium sulphure, to immobilized metallic ions. Samples where then kept at 4 – 8 ° C. After two 1 hour washes in phosphate buffer, the tissue was dehydrated in a graded ethanol series. Root segments where dried to critical point (Sandri 780-A) and mounted on aluminum stubs using double-carbon tape, and observed under a Low Vacuum SEM JEOL JSM-5600LV equipped with an EDS Noran Microscopy central lab of Physic Institute-UNAM The EPT analysis was performed in three regions: cortical (external root), vascular tissue (internal root) and hyphae regions, in an interval of 1400-5000 counts. Metal ions were precipitated as insoluble salts (3) and EPTs were measured as chemical elements. This permitted to establish indirect correlation between EPT-accumulation and localization of ligands as -SH groups. Table 1 shows EPTs concentration (%) in *Casuarina* roots and hyphae colonizing this surface of roots. It is remarkable the presences of Ti and Pb as these elements are toxic to plants even at trace concentrations. Ti concentration in the cortical part of the roots and the hypha regions was 0.22 and 0.18%, respectively, while no content was detected in the internal part (vascular tissue) of the root. Pb was accumulated in the hyphae region (1.24 %), but not at the cortical and vascular part of the root. These observations suggest a protection role from the AMF hyphae to avoid plant toxicity. Some mechanisms involved in Pb exclusion in the root are: biosorption and accumulation at hyphae level, and root absorption restriction. These results support the hypothesis of presence of structural compounds with functional chemical radicals (for example sulfihyl groups with protein origin) trapping Pb and part of Ti at hyphae

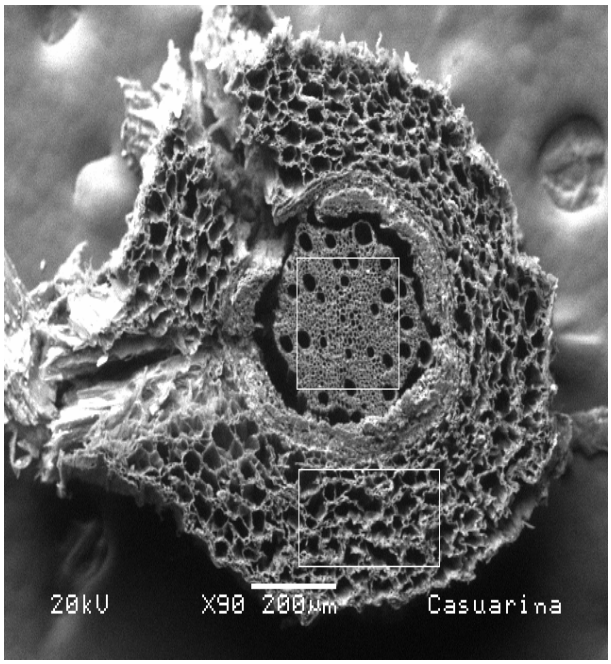
level. Some authors (1,2) demonstrated that external hyphal complex proportionate an absorption surface, either inside and outside of the colonized roots of the host plant to sequestrate and accumulate different EPTs. This mechanism favors exclusion and avoids transport of EPTs into the root system, reducing plant toxicity. The high Fe content also supports the participation of Glomalin, protein produced by hyphae of all AMF, in the sequestration of EPTs (2). From the Table 1, the presence of Zn, Si and Cu in the vascular part of the root, was twice the percentage of those observed in the cortical part of the root and the external hypha. Contrary, Ca content was higher in the cortical part of the root. Si and Ca may be acting as precipitators of different EPTs at internal and external root level.

SEM-EDS were useful techniques to identify *in situ* distribution and concentration of specific EPTs in *Casuarina* spp. roots. The external hyphae of AMF accumulated Pb and avoided Ti translocation into the host plant. Ti was located only in the cortex the external part of the roots, where it appears to be accumulated. These results suggested accumulation and exclusion of EPTs by AMF allowing grow and development of *Casuarina* plants in polluted soils with heavy metals.

Table 1. Percentage of elements in *Casurarina* spp. roots determined by EDS

Element	**Root part**		**External hyphae**
	cortex (external)	**vascular tissue (internal)**	
C	39.15	30.99	37.59
O	29.87	16.60	30.16
Zn	2.47	5.21	2.08
Al	1.48	2.20	2.25
Si	4.03	18.35	4.84
P	0.74	0.71	0.82
S	8.75	4.37	4.28
Mo	0.63	0.66	0.74
K	0.78	6.17	1.74
Ca	4.01	1.67	1.74
Mn	0.14	0.00	0.28
Fe	3.59	7.81	5.88
Na	1.20	0.36	0.69
Mg	0.73	0.24	0.56
Cu	2.21	4.66	1.78
Ti	0.22	-	0.18
Pb	-	-	1.24

Fig. 1. SEM observation of a *Casuarina* spp. root accumulating different elements



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